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The Afferent Nerve Response of the Movement Receptor around the Anal Region of the Crayfish¹⁾

By

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(With 11 Text-figures)

The rhythmical contractile activity was observed in the anus of the crayfish and the lobster by Miller (1910). Such a rhythmical activity of the anus is not caused by the contraction of the sphincter muscle but by the circular muscle and the radial muscle-fibres surrounding the anal cavity (Miller, 1910; Janisch, 1924). Electromicroscopic structures (Whitear, 1960) and the physiological features (Burke, 1954; Pringle, 1956; Wiersma, 1959) of the proprioceptor which reacts to the movement of legs or body segments of the crustacea have been well known. However there is little known concerning the movement receptor of the anus of the crayfish.

It is a very interesting problem whether the regular and constant characters of the anal rhythm is produced by the myogenic mechanism. The intestine of the crayfish is well noticed by its autonomic activity, that is, the peristaltic movement. However the exact origin of this autonomicity is also unknown. Some investigators' suggested the myogenic (e.g. Ebara, 1969) and the other neurogenic nature (Florey, 1954; Prosser *et al.*, 1965).

The present paper is given in order to make clear the existence of the receptor concerned with the anal movement, the structure and the physiological function. In addition an attempt is done to elucidate the origin of the autonomicity of the anal activity.

Material and Methods

Experiments were carried out on 72 male and female crayfishes (*Procambarus clarki* Girard) kept in the laboratory tanks. Room temperature during the experiments was 19°-25°C.

Histology: The carapace of the dorsal side of the anus was removed and a piece of the anal part was isolated and the isolated anus was fixed for 48 hours in the solution (formalin 5 cc+acetic acid 5 cc+80% alcohol 90 cc). If the crayfish was immediately after

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the ecdysis, it was used without the removal of the carapace. After fixation, the isolated anus was embedded into the paraffine block. Serial section ($10\ \mu$ in thickness) were prepared and stained with the Bodian method modified by Otsuka (1962), because this method is good for the detection of the nervous structure.

In addition the isolated anus was also fixed for 2 hours with Bouin solution (saturated picric acid sol. 75 cc + 95% formalin 25 cc + acetic acid 5 cc) and serial section ($10\ \mu$ in thickness) were stained with eosin and acid-haemalaum to know the histological structure of the anal part and the anal movement receptors.

Preparation: A piece of carapace was removed carefully at the dorsal side of the anus. This procedure was made it easy to cast the light through the anal cloaca and to monitor the anal contracting movement. Then the crayfish was fixed dorsal side down in the chamber filled with cooled crayfish Ringer's solution buffered at pH=7.2 with Na-bicarbonate (van Harreveld, 1936). To prevent the blood flow the blood ducts situated between the posterior abdominal ganglion and the anus were tied with a fine nylon thread ($50\ \mu$ in diameter). Since the blood flow around the anal part was very little on the abdomen preparation which was obtained from the separation between the thorax and the abdomen, the blood ducts were not tied as the case of the whole body preparation. Under the binocular microscope ($24\times$), a fine nylon thread ($50\ \mu$ in diameter) was pierced through a hole on the central part of the anal lip made by a needle and the anal lip was tied with this thread. The passive movement of the anal lip was caused by pulling this thread left and right (opening or closing movement) or upwards and downwards.

The anal compressor muscles of the both sides were removed, then the anal nerve and the intestinal nerve were detected as was shown in Figure 1, A. The anterior nerve, the uropod nerve and the telson nerve were cut off at the proximal part of the posterior abdominal ganglion to prevent the interference of the another units other than the anal movement receptors. The active autonomic activity of the anus consisted of successive movements in which first the opening movement of the anal lip occurred and then the contractile activity of inside wall of cloaca followed. So the passive movement of the anus only produced the movement of the anal lip but it could not cause the contractile activity of the inside wall of the cloaca.

To examine the inherent autonomic nature of the anus itself, the isolated anus preparation was also prepared in which all connexions between the anus and the central nervous system were removed, that is, all nerves were cut off at the proximal part of the posterior abdominal ganglion on the abdomen preparation.

Stimulation and recording: To monitor the opening and closing movement of the anus, the phototransistor (Toshiba, OSI4) was set just under the position of the anal part of the crayfish, and a lamp (a light source of a microscope was used) was arranged above the cloaca of the anus. The output of the phototransistor was led to another channel of the oscilloscope (Nihonkoden type, VC-7) through the AC-amplifier. The active autonomic activity of the anus was produced by application of electrical stimuli to the posterior intestinal nerve. The repetitive pulses (1 msec in duration, 0.3–10 V in strength) produced by a square pulse generator were fed to a pair of stimulating electrodes of Ag-AgCl type.

The action potentials of nerve were recorded by an electrode of Ag-AgCl type which was held with a micromanipulator and were led to another channel of the oscilloscope, and recorded by a long-recording camera. As an indifferent electrode, a silver plate was put under the animal and was connected with earth.

A black fine glass rod (1.5 mm in diameter) was used for tactile stimulation of the anus or the body. By using the black rod, a signal of tactile stimulation could be detected with the similar arrangement to the anal movement.

Results

I. *Anatomy*: The schematic appearance of the innervation around the anal part was shown in Figure 1, A. Three anal movement receptors were detected and they were termed as the vertical anal movement receptor (VAMR), the horizontal anal movement receptor (HAMR) and the telson tactile receptor (TTR). i) VAMR was composed of the radial muscle-fibres (r.m.) spanned vertically between the dorsal lip of cloaca and bottom of the dorsal carapace of the telson as was shown in Figure 1, B. The sensory cell bodies were observed on the mucous membrane (m.m.) of the anus and the process of these cells ran to both directions of the cloaca and r.m., that is, they were bipolar cells (Fig. 1, C). The process of the sensory cell bodies through r.m. joined to the anal nerve (an.n.). The sensory cell other than that was present on the m.m. was not detected on r.m. . ii) HAMR was composed of the radial muscle-fibres (r.m.) around the anal cloaca spanned radially to the horizontal direction as was shown in Figure 1, A and B. The one end of r.m. was connected with m.m., and the bipolar cell bodies was also situated on this membrane similar to the VAMR. The process of the cell bodies ran through r.m. and ascended to the posterior abdominal ganglion (p. abd. g.) as an afferent nerve (an. 2) together with the anal nerve (an.n.). The clear striation was observed on r.m. of VAMR and HAMR. In addition the circular muscle and the longitudinal muscle of the intestine were also striated. On the part of cloaca the longitudinal muscle was disappeared and only the circular muscle was observed. iii) TTR was present between the posterior telson flexor muscle (p. t. f. m.) and the ventral telson flexor muscle (v. t. f. m.) as was shown in Figure 1, B. The afferent nerve (an. 3) was situated among these muscles and reached to the lateral outside of v. t. f. m. . The sensory cell bodies on the connective tissue between lateral outside of v. t. f. m. and the anus could not be found, but it seems that they will exist on the lateral outside of v. t. f. m. from the methylene blue staining preparation. The afferent an. 3 nerve also together with the anal nerve ascended to p. abd. g. similar with the afferent nerves of the VAMR and the HAMR.

These three receptors mentioned above reacted to the anal movement and they send their afferent informations to p. abd. g. . On the other hand, the efferent information to control the anal activity from the p. abd. g. was sent by the anal motor nerve (an. m. n.) and an intestinal nerve (int. n.). The former ramifies to the anal compressor muscle (an. c. m.), anal dilator muscle (an. d. m.), anterior telson flexor muscle (ant. t. f. m.), posterior telson flexor muscle (p. t. f. m.) and ventral telson flexor muscle (v. t. f. m.) as is shown in Figure 1, A. According to Keim (1915), the anal motor nerve and the anal nerve were composed of the common nerve bundle on *Astacus fluviatilis* L., but on *Procambarus clarki* these both nerves were clearly separated. The muscle an. c. m. and an. d. m. attach to the rostral side of the anal margin and will control the movement of the anal lip (an. l.). However these muscles are not directly connected with an. l. and so they will incidentally move an. l. .

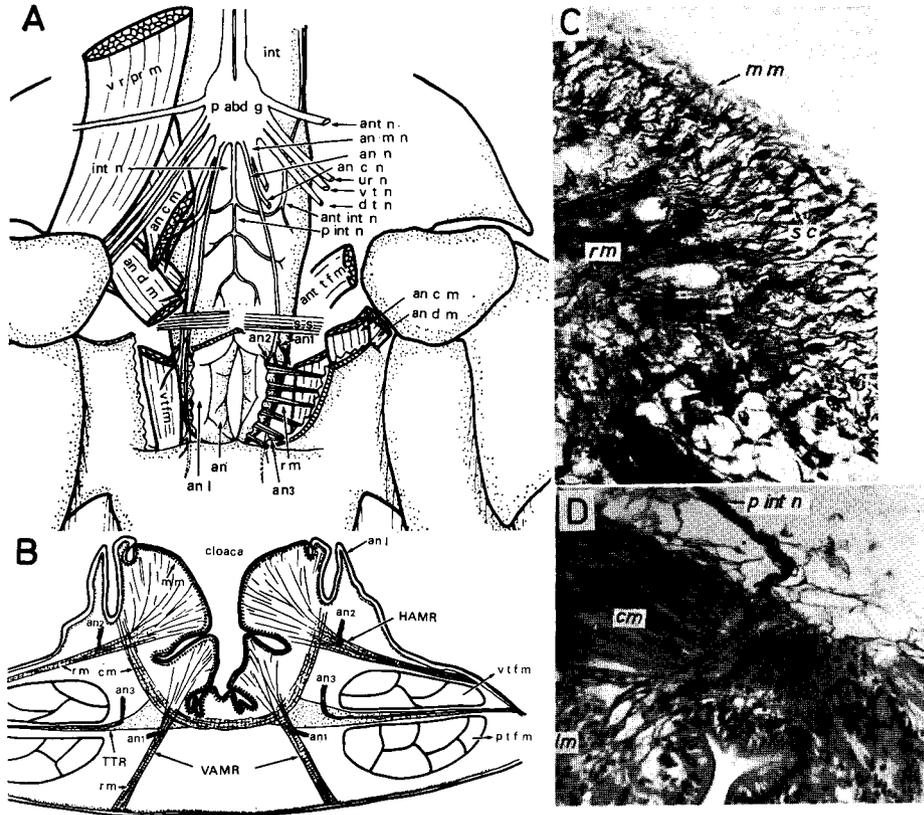


Fig. 1. Schematic representation showing the innervation around the anal part (A) and the structure of the anal movement receptors (B). The microphotograph of the anus (C) and the rectum (D).

A) Ventral view of the innervation from the posterior abdominal ganglion to the anal part and the intestine. *int*, intestine; *v r pr m*, ventral rotator protopodite muscle; *p abd g*, posterior abdominal ganglion; *ant n*, anterior nerve; *an m n*, anal motor nerve; *int n*, intestinal nerve; *an n*, anal nerve; *ur n*, uropod nerve; *v t n*, ventral telson nerve; *d t n*, dorsal telson nerve; *ant int n*, anterior intestinal nerve; *p int n*, posterior intestinal nerve; *an c m*, anal compressor muscle; *an d m*, anal dilator muscle; *ant t f m*, anterior telson flexor muscle; *s s*, stretch structure; *an 1*, an afferent branch to VAMR; *an 2*, an afferent branch to HAMR; *v t f m*, ventral telson flexor muscle; *an*, anus; *r m*, radial muscle-fibres; *an 3*, an afferent branch to TTR.

B) Scheme of transverse section through middle of anus showing the position and the structure of anal movement receptors. *an 1*, anal lip; *m m*, mucous membrane; *HAMR*, horizontal anal movement receptor; *c m*, circular muscle; *TTR*, telson tactile receptor; *VAMR*, vertical anal movement receptor; *v t f m*, posterior telson flexor muscle; *an 1*, *an 2*, *an 3*, *an 1*, *v t f m* and *r m*, see the explanation of (A).

On the other hand, the contraction of the inside wall of cloaca will be modulated by int. n. . The nerve int. n. is given from p. abd. g. to the rectum. It divides into the three branches and the two of which are distributed anteriorly along the both sides of intestine (anterior intestinal nerve=ant. int. n.), while the other (posterior intestinal nerve=p. int. n.) passes posterior towards the anus and the latter distal process was observed to penetrate into the stretch structure (s. s.) and the circular muscle of the rectum as was shown in Figure 1, D. The stretch structure was similar histological structure to r.m., but the connexion with r.m. and its function were not clear.

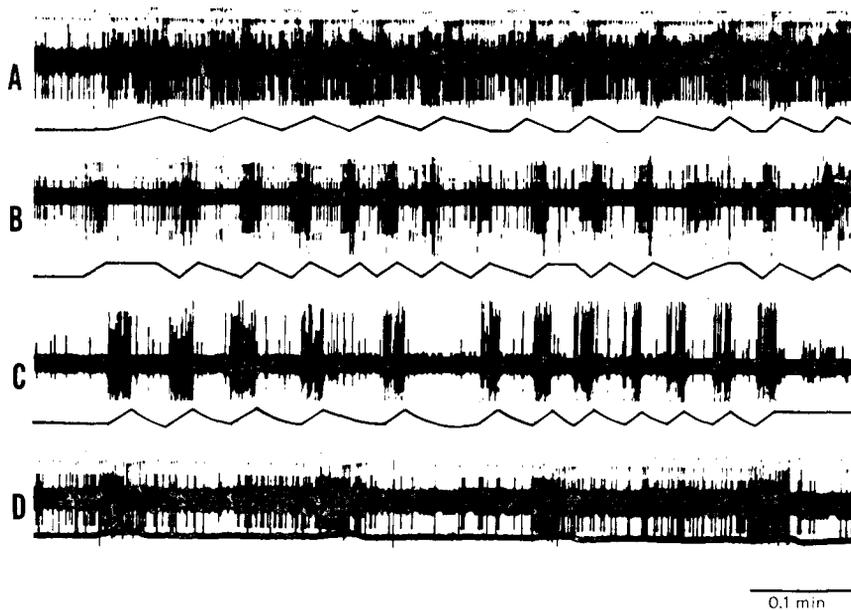


Fig. 2. Afferent discharge responses of the vertical anal movement receptor (VAMR) to passive opening and closing of the anal lip (A and B), passive pulling the anal lip upwards and downwards (C) and active contractile activity of the anus (D). Upper trace indicates impulses of VAMR and lower trace indicates the movement signal of the anal lip; opening upwards in A, B and D, closing downwards in A, B and D, pulling upwards in C, pulling downwards in C.

Fig. 1.

C) Microphotograph of transverse section of the middle part of the anus showing the bipolar sensory cells (s c) and the radial muscle-fibres (r m). Staining silver solution by Bodian method.

D) Microphotograph of transverse section of the rectum representing the posterior intestinal nerve (p int n) innervating the circular muscle (c m) and the longitudinal muscle (l m) of the intestine. Staining, eosin and acid-haemalum.

II. *Physiology*: Discharge responses of VAMR, HAMR and TTR to the active rhythmical contractile activity of the anus, to the passive movement of the anal lip or to tactile stimulation were recorded from an. 1, an. 2 and an. 3, respectively, in order to know their functions.



Fig. 3. Afferent discharge responses of VAMR (A and B) and HAMR (C and D) to tactile stimulation of the anus, and a train of impulse burst observed after tactile stimulation (D and E). A and B, responses of VAMR to tactile stimulation of the central part and the posterior part of the anus, respectively; C and D, responses of HAMR to tactile stimulation of the central part and the posterior part of the anus, respectively; E, response observed on the anal nerve that contains the responses of VAMR, HAMR and TTR showing a remarkable train of impulse burst. Upper trace indicates the action potentials and lower trace indicates the signal of tactile stimulation, and in order to recognize easily the duration of tactile stimulation an underline was drawn below the each lower trace.

A) *Response of the VAMR*. Passive closing movement of the anal lip elicited a burst of large impulses in an. 1 of VAMR as was clear from Figure 2, record A. This large impulse fired only to passive closing but not to opening and its response sustained during the closing movement. On the other hand, passive opening produced the burst of the largest, median and small impulses as was shown in Figure 2, records A and B. The response of the median impulse was continued during the opening movement (Fig. 2, B), while that of the largest impulse was phasic. Concerning to the active contractile activity the similar response to the passive movement was observed, however a burst of the largest

impulse was not observed in this case.

When the anal lip was pulled upwards and downwards, to rostral and caudal direction, respectively, the VAMR was more sensitive to the upward movement than the downward one (Fig. 2, C).

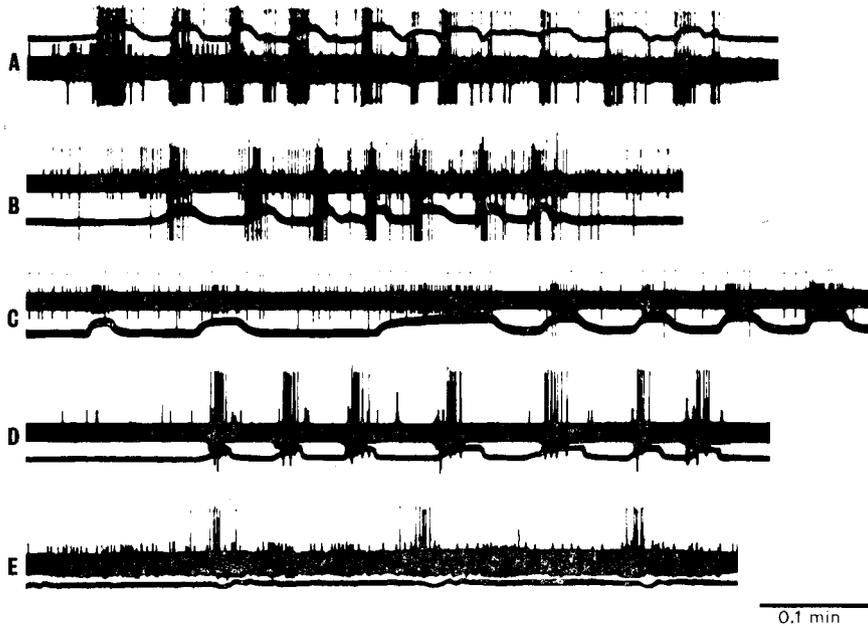


Fig. 4. Afferent discharge responses of horizontal anal movement receptor (HAMR) to passive opening and closing of the anal lip (A and B), to passive pulling the anal lip upwards (C) and downwards (D), and to the active contractile activity of the anus (E). Upper trace of B, C, D and E, and lower trace of A mean action potentials. Lower trace of B, C, D and E, and upper trace of A mean the movement signals; opening upwards in A and B, closing downwards in A and B, pulling the anal lip to the rostral direction upwards in C, pulling the anal lip to the caudal direction downwards in C and D, opening downwards in E, closing upwards in E.

And also the VAMR was very sensitive to tactile stimulation of the anus, that is, the central part of cloaca (Fig. 3, A) and caudal part of cloaca (Fig. 3, B). The different kinds of fibres fired to tactile stimulation according to the different stimulated parts. It is proved that VAMR reacts as a movement and tactile receptors, but it never responds to tactile stimulation of parts other than the anus.

B) *Response of the HAMR.* Similar to VAMR, HAMR was very sensitive to the tactile stimulation to the anal part, more sensitive to the caudal part of anus (Fig. 3, D) than to the central part (Fig. 3, C). Usually the one to one response to tactile stimulation of the anus was found, however sometimes a train

of impulses was set off after tactile stimulation as was shown in Figure 3, record D. As is clear from Figure 3, record 4, such a train of impulses was very conspicuous when the record was obtained from the anal nerve containing the nerves of an. 1, an. 2 and an. 3. Such a typical impulse burst will imply that it will be set off from the primary sensory neurons.

When the anal lip was passively opened, a strong burst of the large impulses was observed on an. 2 of HAMR and it could not be observed to the passive closing movement (Fig. 4, A and B). On the other hand, passive closing movement of the anal lip caused a burst of the small impulses (Fig. 4, B). HAMR was more sensitive to passive opening than to passive closing.

When the anal lip was pulled downwards, the phasic response of the large impulse was observed (Fig. 4, C), while the sustaining response of the small impulses was observed to upward movement (Fig. 4, D). HAMR was more sensitive to the downward movement than to the upward movement.

When the autonomic contractile activity was found out, a typical difference in responses was not observed compared with that to passive movement as was clear from Figure 4, record E.

The tactile stimulation to the left lip produced a strong burst only on an. 2 of the left side, while the tactile stimulation to the right lip produced a strong burst only on an. 2 of the right side, when the anal lip was separated left and right at the central line. It seems that the left and the right an. 2 react to the different sensitive field on the anal lip and its field is never overlapped. This was true in an. 1 of VAMR.

C) *Response of the TTR.* When the anal lip was passively closed, the sustaining response of small impulses was observed on an. 3 of TTR (Fig. 5, A). On the other hand, passive opening movement caused a burst of large impulses, but TTR was more sensitive to passive closing movement than to passive opening movement. When the anal lip was pulled upwards, a strong burst of small impulses was found, while to downward movement the TTR was not so sensitive as was clearly shown in Figure 5, record B. HAMR and VAMR never reacted to tactile stimulation of the telson or the uropod, but the TTR was very sensitive to tactile stimulation of these parts (Fig. 5, C).

D) *Efferent responses of anal compressor and dilator muscles.* The anal motor nerve (an. m. n.) was completely efferent, since no firing of this nerve was occurred after it was severed at the proximal part of p abd. g.. The nerve an. m. n. sends branches to the anal compressor and anal dilator muscles, and efferent responses of these muscles were recorded from each efferent nerve to anal movement and to tactile stimulation. Always a discharge response of high frequency (19-21 impulses/sec) was observed on the efferent nerve of the anal dilator muscle. However if the anal lip was passively opened or closed, this spontaneous impulse burst was completely blocked as was shown in Figure 6, record A. This blockage of spontaneous activity was continued for more than 8 sec during the movement. In addition similar blocking phenomenon was also observed

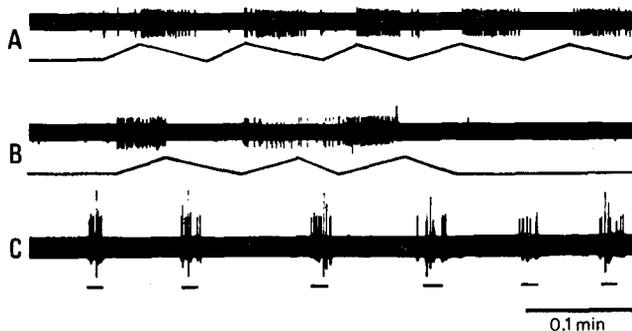


Fig. 5. Afferent responses of TTR to passive opening and closing of the anal lip (A), to passive pulling the anal lip upwards and downwards (B) and to tactile stimulation of the uropod and the telson (C). Upper trace indicates the response impulses and lower trace signals of stimulation; opening upwards in A, closing downwards in A, pulling the anal lip to the rostral direction upwards in B, pulling the anal lip to the caudal direction downwards in B, underlines mean the duration of the tactile stimulation.

to tactile stimulation of the anus, but it was soon adapted within 2 sec and again a spontaneous activity occurred as was clear from Figure 6, record B.

On the other hand, when the autonomic contractile activity occurred in the anus, no blocking phenomenon occurred and no reflex response was observed on the efferent nerve of anal dilator muscle (Fig. 6, C).

Also on the efferent nerve of anal compressor muscle the blockage of spontaneous activity (11–16 impulses/sec) was observed to passive movement of the anal lip (Fig. 6, D). But this blockage was not so clear compared with that on the efferent nerve of the anal dilator muscle and it was not always observed, in most cases no response was produced by passive movement of the anal lip. The blockage to passive movement lasted more than 1.5 sec. To tactile stimulation also blockage of spontaneous activity was observed, but it was not so clear than that of the anal dilator muscle. On the other hand, the active movement of the anus did not produce the reflex response on the efferent nerve of the anal compressor muscle similar to that of the anal dilator muscle (Fig. 6, E).

As soon as the input information of the anal nerve was omitted by severing the anal nerve, the blocking phenomenon mentioned above was not observed at all on these both muscles. This fact means that a reflex arc, that is, anal movement receptors→p. abd. g.→anal dilator and compressor muscles, presents.

E) *Control of rhythmical anal contractile activity.* A synchronous contractile activity was always observed before preparation. The tactile stimulation of the mouth part always caused the rhythmical activity and it sometimes occurred in response to tactile stimulation of the abdomen. In addition when the abdomen preparation or the anus preparation was made by separation between the abdomen and the thorax or by cutting all connexions between the

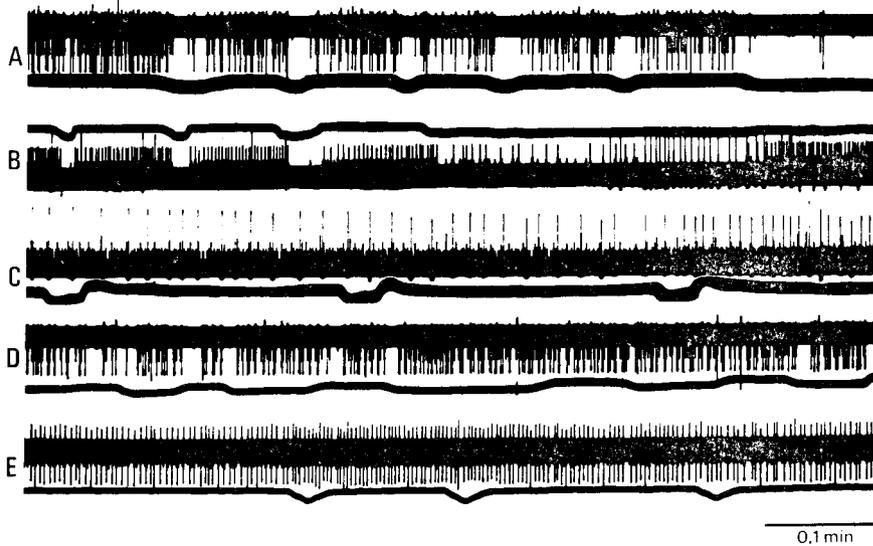


Fig. 6. Efferent reflex responses of anal dilator (A, B and C) and anal compressor muscles (D and E) to passive opening and closing movement of the anal lip, to tactile stimulation of the anus (B) and to the active rhythmical movement of the anus (C and E). Upper trace of A, C, D and E, and lower trace of B indicate the reflex discharge impulses. Lower trace of A, C, D and E shows a movement signal; opening downwards and closing upwards.

central nervous system and the anus, respectively, a contractile activity was always produced 3–8 times. In some cases (30 among 72 crayfishes, 40%), the rhythmical activity lasted more than 3 min, but in other cases (38 among 72 crayfishes, 53%), it soon stopped after preparation. In a few cases (4 among 72 crayfishes, 5%), the activity was observed for a long time on the anus preparation, especially in 2 cases it lasted more than 5 min.

The rate of the rhythm was closely related to the freshness of the animal. Namely it was 6–8 times in a min in the old crayfish kept more than 1 month in laboratory tanks, while 12–14 times in a min in the fresh one obtained in the early summer. The time required during opening and closing movement was 2–3 sec in the former old crayfish, while within 1 sec in the latter fresh one. No precise difference in the rate and the behavior of the contractile activity was observed among preparations, that is, whole body, abdomen and anus preparations.

The repetitive electrical stimulation of the nerve cord produced sometimes, but not always the contractile activity. Same was true in the case of the electrical stimulation of the mid-intestine. However the electrical stimulation of the posterior intestinal nerve always caused the rhythmical activity of the anus. The electrical stimulation of the anterior intestinal nerve caused the peristaltic

movement of the intestine as was observed by Miller (1910), but no obvious effect was given to the contractile activity of the anus.

F) *Response of the anterior intestinal nerve (ant. int. n.).* The nerve ant. int. n. always fired spontaneously at the frequency of 9 impulses/sec (Fig. 7, A). When the anal lip was passively opened or closed, the spontaneous firing of impulses was not changed at all as was clear from Figure 7, record B. Same was true in the active contractile movement (Fig. 7, C). In addition no remarkable difference was found in the response of impulses before (Fig. 7, D) and after (Fig. 7, E) cutting

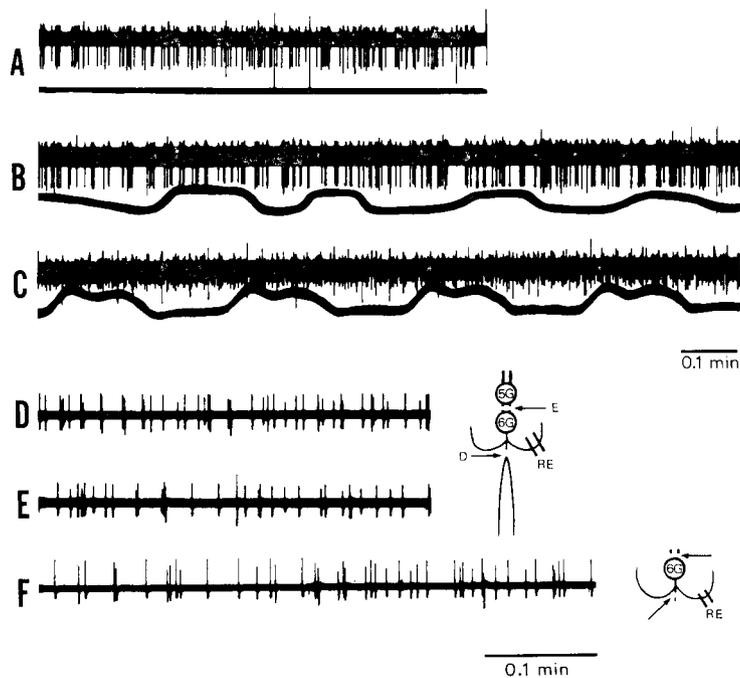


Fig. 7. Discharge responses of the anterior intestinal nerve (ant. int. n.). A, spontaneous activity on the intact preparation; B, response to passive movement of the anal lip (opening downwards and closing upwards of the lower trace); C, response to the active contractile activity of the anus (opening upwards and closing downwards of the lower trace); D, response after severing the distal part of ant. int. n. and the position of severing ant. int. n. (arrow D) and a recording electrode (RE) is shown in the figure on the right side; E, response after cutting the nerve cord between the 5th abdominal ganglion (5G) and the posterior abdominal ganglion (6G) as is shown in the figure on the right side (an arrow E indicates the position of the cutting part and RE a recording electrode); F, response 5 min after removal of all connection other than 6G as is shown in the figure on the right side (arrow indicates the position of the cutting of the nerve and RE means the position of the recording electrode). A, B, C and D, E, F are successive records from the same two preparations, respectively.

the connective between the 5th abdominal ganglion (5G) and p. abd. g. (6G). Moreover even when p. abd. g. and ant. int. n. were isolated from the crayfish schematically shown in the right side of Figure 7, record F, the spontaneous firing was still observed and it lasted more than 5 min (Fig. 7, F). Miller (1910)

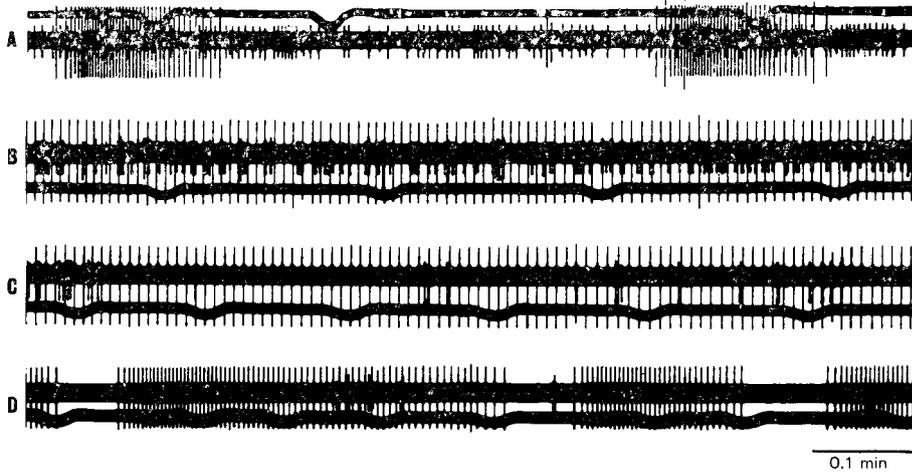


Fig. 8. The discharge response of the posterior intestinal nerve to active rhythmical contractile activity of the anus showing the relationship between the frequency of a train of impulses and the rate of the contractile activity. Upper trace of A and lower trace of B, C and D represent a movement signal of the contractile activity; opening downwards and closing upwards. Lower trace of A and upper trace of B, C and D represent the discharge responses of p. int. n. A, B, C and D are records from four different preparations, respectively.

suggested the possibility of the efferent and afferent nature of this nerve, however from these observations mentioned above it proves that ant. int. n. is a completely efferent nerve and it will receive its efferent information from p. abd. g. but from a higher level of central nervous system than p. abd. g. The response of ant. int. n. did not change when the electrical stimulation was given directly to the mid-intestine. This also emphasizes this conclusion that the nerve is efferent. No clear relationship between the discharge response of ant. int. n. and the rhythmical contractile activity of the anus was found in the present experiment.

G) *Response of the posterior intestinal nerve (p. int. n.).* When the anus contracted regularly and spontaneously, a train of large impulses was observed on p. int. n. as was clear from Figure 8. However no direct correlation between this train of impulses and the anal activity was found as clearly shown in Figure 8. But it was generally observed that the duration between each large impulse becomes shorter, the more increases the rate of the anal contractile activity.

When the repeated electrical stimulation (1 msec in duration, 0.3-10 V in

strength) was given to p. int. n., the rhythmical activity was often produced. When the frequency of stimulation was changed, the rate of the rhythmical activity elevated in accordance with the increase of the frequency (Fig. 9). When the frequency was increased to more than 50 Hz the anal rhythm of constant

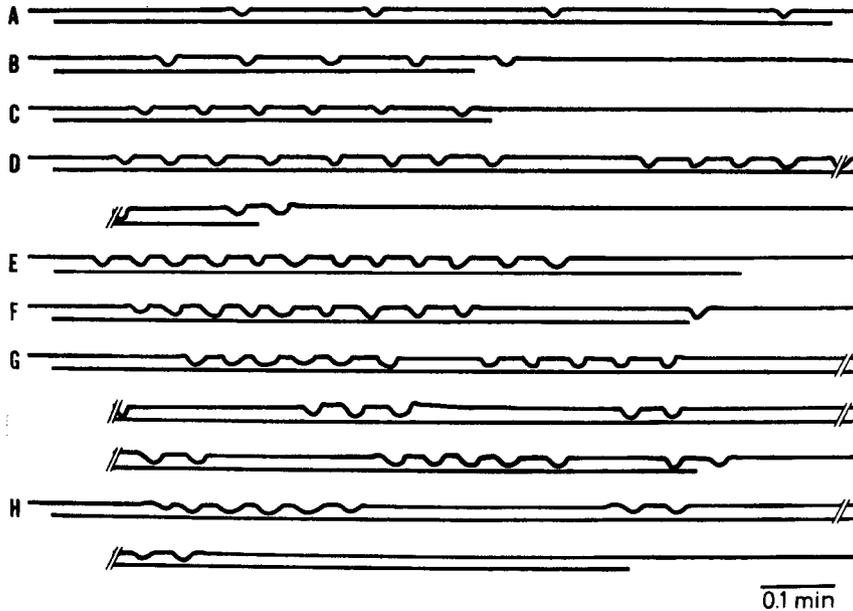


Fig. 9. Anal contractile activity produced by repetitive electrical stimulation to a posterior intestinal nerve showing the relationship between the frequency of the stimulation and the rate of the contractile activity. A, contractile activity at the frequency of 3 Hz; B, 10 Hz; C, 20 Hz; D, 30 Hz; E, 40 Hz; F, 50 Hz; G, 60 Hz; H, 70 Hz. Upper trace represents a movement signal of the contractile activity; opening downwards, closing upwards. Underlines mean the duration of repetitive stimulation.

character tended to decay as was clear from Figure 9, records F, G and H. The rate of the contractile activity observed in the present experiment was 6–8 times in a min or 12–14 times in a min. These corresponded with the rate of the activity to the stimulation of the frequency within 30 Hz. On the other hand, when the frequency of the electrical stimulation was kept constant (30 Hz) and the strength was changed to twice (Fig. 10, B), three times (Fig. 10, C), four times (Fig. 10, D) and five times (Fig. 10, E) of the initial strength (Fig. 10, A), the rate of the contractile activity was increased in accordance with the increase in the strength of the electrical stimulation. However too much increased the strength was, the rate of response decreased (Fig. 10, E) and the constancy of the anal rhythm was also lost. From above results obtained by changing the frequency and the strength of the repetitive

electrical stimulation of p. int. n., it proved that the frequency was more important factor to cause the rhythmical activity than the strength factor was. And also these results may induce the possibility that a train of large impulses observed on spontaneous contractile activity of the anus (see, Fig. 8) will be efferent response of p. int. n. to modulate the anal activity. However the fact

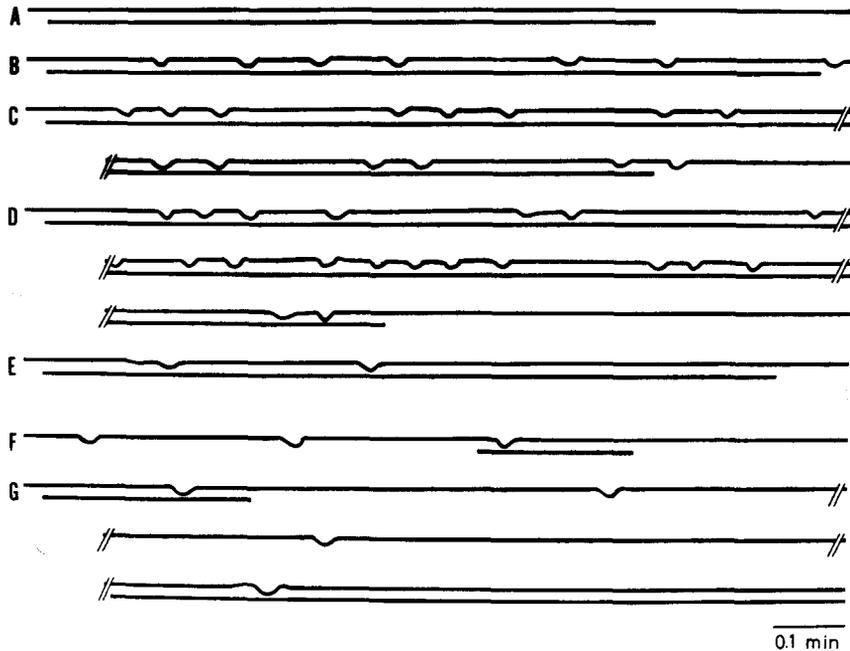


Fig. 10. Anal contractile activity produced by the repetitive electrical stimulation (0.3–10 V in strength, 30 Hz in frequency and 1 msec in duration) to p. int. n. in accordance with the difference in the strength of the stimulation (A, B, C, D and E) and the effect of the repetitive electrical stimulation applied to p. int. n. during or after spontaneous contractile activity (F and G). Upper trace indicates the movement signal of contractile activity; opening downwards and closing upwards. Underlines indicate the duration of repetitive electrical stimulation. The strength of the stimulation in B, C, D and E is twice, three times, four times and five times of that in A, respectively.

looked like more complicate than that was expected. Although this train of large impulses was observed on p. int. n., in some case no activity was produced and moreover the rhythmical activity was occurred even on the anus preparation in which all connexion between the central nervous system and the anus was removed.

In addition a train of impulses was also observed after cutting the proximal part of the intestinal nerve (Fig. 11, A) and this response was not changed by the passive movement of the anal lip (Fig. 11, B). A strong burst elicited in p. int. n.

to tactile stimulation of the anus on the anus preparation (Fig. 11, C) and also in this anus preparation the discharge response to the active contractile activity was observed on this nerve as was clearly shown in Figure 11, record D. These facts will suggest that p. int. n. is afferent and efferent at the same time.

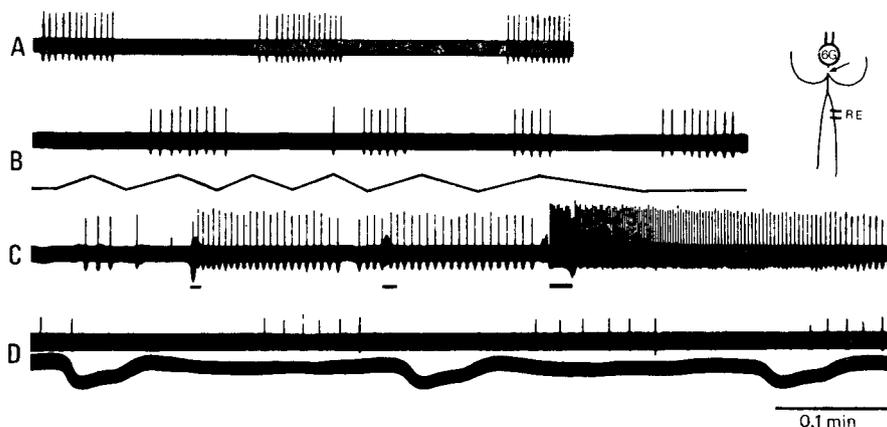


Fig. 11. Discharge responses of a posterior intestinal nerve to passive movement of the anal lip (B), to tactile stimulation of the anus (C) and to active contractile activity of the anus (D) on the isolated anus preparation. A is the spontaneous burst of a posterior intestinal nerve. The figure on the upper right side represents the position of the recording electrode (RE) and the cutting position of the nerve (an arrow) on the isolated anus preparation. Lower trace of B represents the movement signal, opening upwards and closing downwards. The underline of C means the signal of the tactile stimulation. Lower trace of D means the movement signal of the active activity of the anus; opening downwards and closing upwards. A, B and C are successive records from the same preparation.

Discussion

It was observed that propodite-dactylopodite (PD) organ consisted of the elastic strand with clear striations and the sensory cell bodies was embedded in this strand (Muramoto and Murayama, 1965). Most of the joint receptor is similar structure to the PD organ (Pringle, 1956; Wiersma, 1959; Cohen, 1963). However on the anal movement receptors (VAMR and HAMR) the sensory cell bodies did not exist in the radial muscle-fibres, but they existed only at the one end of the radial muscle-fibres attached to the mucous membrane of the anus. These cells are bipolar neurons similar to those of the joint receptor. The fact that the sensory cell bodies were present on the mucous membrane will coincide with the fact that the anal movement receptors were more sensitive to tactile stimulation of the anus than to the contractile activity of the anus. In addition it will also be suitable for the perception of the existence of the excrements in the anus.

It proves that VAMR and HAMR are the proprioceptors of the contractile activity of the anus from the anatomical and physiological results obtained in the present study. But TTR seems to act mainly as tactile receptor, because it is not so closely connected with the anal activity from its anatomical point of view. Moreover it is very sensitive to the tactile stimulation more than to the anal activity. In addition the response of the TTR to passive movement of the anal lip may be caused by the tactile stimulation produced by the passive movement. When the response is recorded from an. 3 of TTR, always it needs to isolate the anal lip. So spontaneous contractile activity does not appear at all and the response to the spontaneous activity can not be obtained. This will emphasize the possibility that the TTR is mainly a tactile receptor.

Miller (1910) suggested that the peristaltic contraction is conducted to the anus by means of the circular muscle layer of the intestine, and the arrival of peristaltic wave from the rectum initiates the first opening movement of the anal rhythm. However in the present experiment it could not be found out that the peristalsis in the rectum caused the contraction of the anus, but it was always observed that the peristalsis followed the rhythmical activity of the anus and never vice versa. This fact will be proved also from the following points. When the isolated crayfish intestine kept in van Harreveld's solution, the peristaltic activity can be observed even after 24 hours. But anal contractile activity does not last more than 4 hour in the abdomen preparation and this activity can not be observed only in almost half cases (53%) of all preparations.

Floreay (1954) inferred the existence of an excitatory and inhibitory innervation from his pharmacological experiments and Prosser *et al.* (1965) and Ebara (1969) noted the existence of a pacemaker to regulate the peristaltic movement of the intestine. It will be probable that the complicated nervous connexion will also exist between the peristaltic movement and the rhythmical anal activity. In fact the rhythmical activity of the anus was still observed on the anus preparation in which all connexion between the central nervous system and the anus was removed. This fact renders it probable that a pacemaker concerned in the production of the anal rhythm does exist around the anal part. It will be also considered that there exist a coordinating mechanism between a pacemaker related to peristaltic movement and a pacemaker concerned with the rhythmical activity of the anus.

The existence of a pacemaker around the anal part was examined by means of the histological method. Finally no neuron around the anus could be found other than the sensory cell bodies on the mucous membrane of the anus. It is probable that these sensory cells are pacemakers to regulate the anal activity and at the same time they act as movement receptor. Similar phenomenon has been reported on a pacemaker of a heart of the crustacea, that is, a pacemaker concerned with the autonomic movement of the heart acts as the receptive elements and both afferent and efferent limbs of the intrinsic reflex may lie within the same neuron (Maynard, 1960). And the impulse burst was observed on

p. int. n. to tactile stimulation of the anus after severing the proximal part of p. int. n. as was shown in Figure 11. This response may be elicited in a pacemaker existed on the mucous membrane. The electrical stimulation to p. int. n. always produced the contractile activity and when the proximal part of p. int. n. was severed, always the anal rhythm was produced. These facts will suggest that p. int. n. has a share in excitation and inhibition of the pacemaker to regulate the anal activity.

According to Orlov (1926), ant. int. n. makes a synapse with the descending process of a superior stomato-gastric nerve on the mid-intestine and this synapse sends its process to posterior intestine. If so, very complicated coordinating mechanism will be expected between the peristalsis of the intestine and the rhythmical activity of the anus. When the repeated electrical stimulation or the tactile stimulation was given to the mid-intestine, the rhythmical activity of the anus was sometimes observed but not always. This will indicate that the descending process originated from the synapse existing on the mid-intestine is also concerned with the anal activity. Further study will have to be performed to make clear the mechanism of the autonomic activity of the anus.

The difference in a rate of the rhythmical activity of the anus was observed between a fresh crayfish (12–14 times in a min) and an old one (6–8 times in a min). This will be related to the change of the feeding condition between in the normal circumstance and in the laboratory tank. The fact that the variation in the rate of the anal rhythm was not detected will also emphasized the above suggestion.

In the present study it proved that there exists a reflex arc, anal movement receptor→p. abd. g.→ anal dilator and anal compressor muscles. But it is unknown what kind of responses are caused in the anal movement by the blockage of spontaneous impulses of anal dilator and anal compressor muscles to the passive movement of the anal lip or to the tactile stimulation of the anus. To know this it need to analyze the electromyogram of these both muscles, and it is intended to investigate and report on the regulating mechanism of both muscles to the opening and closing movement of the anal lip and to the rhythmical activity of the anus in another place.

Summary

The anatomical and physiological studies were performed on the rhythmical contractile activity of the anus of the crayfish, *Procambarus clarki*. The rate at which the anal contractions occur is 12–14 times in a min on the fresh crayfish and 6–8 times in a min on the old one kept for a long time in the laboratory tank. The existence of the anal movement receptors, termed as the vertical anal movement receptor (VAMR), the horizontal anal movement receptor (HAMR) and the telson tactile receptor (TTR), was clarified anatomically and physiologically. And the innervation around the anus of the crayfish was made clear as was shown in Figure 1, A.

1. The contractile movement of the anus is produced by the radial muscle-fibers and the circular muscle. All these muscles including the longitudinal muscle of the intestine are clearly striated.

2. VAMR and HAMR consist of the radial muscle-fibres spanned vertically and horizontally around the cloaca, respectively. The bipolar sensory cell bodies are present on the mucous membrane of the anus and they send their processes to the cloaca and to the radial muscle-fibres, the latter process joins to the anal nerve and reaches at the posterior abdominal ganglion.

3. VAMR was more sensitive to the passive closing movement of the anal lip than to the passive opening and it was more sensitive to pulling the anal lip upwards than to pulling it downwards. And also it was very sensitive to the tactile stimulation of the anus.

4. HAMR responded to passive opening movement more than to passive closing and it was more sensitive to the downward movement than to the upward movement of the anal lip. Similar to VAMR, HAMR was also very sensitive to the tactile stimulation of the anus.

5. The distinct difference was not observed in the response of VAMR and HAMR to the active rhythmical contractile activity of the anus compared with that to the passive movement.

6. TTR was more sensitive to the passive closing movement than to the passive opening movement of the anal lip and it responded to pulling the anal lip downwards, but was not so sensitive to pulling it upwards. In addition it was very sensitive to the tactile stimulation of the uropod and the telson.

7. It was proved that there existed the reflex arc, that is, the anal movement receptor→posterior abdominal ganglion (p. abd. g.)→anal dilator and anal compressor muscles.

8. An anterior intestinal nerve was an efferent nerve that receives the information from p. abd. g. .

9. The repetitive electrical stimulation to a posterior intestinal nerve often caused the rhythmical contractile activity of the anus. The rate of the rhythm was closely related to the frequency of the electrical stimulation than to the strength of it.

10. In the isolated anus preparation in which all connexions between the anus and the central nervous system was removed, the rhythmical activity of the anus lasted for more than 5 min. From this fact it was suggested that a pacemaker concerned with the autonomic activity of the anus will be present around the anus. The nature of this pacemaker and the origin of the autonomic activity of the anus was discussed.

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References

- Burke, W. 1954. An organ for proprioception and vibration sense in *Carcinus maenas*. J. Exp. Biol. **31**: 127-139.
- Cohen, M.J. 1963. The crustacean myochordotonal organ as a proprioceptive system. Comp. Biochem. Physiol. **8**: 223-243.
- Ebara, A. 1969. Spontaneous activity of crayfish intestine. Ann. Zool. Jap. **42**: 169-175.
- Florey, E. 1954. Über die Wirkung von Acetylcholin, Adrenalin, Nor-Adrenalin, Faktor I und anderen Substanzen auf den isolierten Enddarm des Flusskrebsees *Cambarus clarki* Girard. Zeitschr. vergl. Physiol. **36**: 1-8.
- Janisch, E. 1924. Der Bau des Enddarms von *Astacus fluviatilis* (*Potamobius astacus* L.). Ein Beitrag zur Morphologie der Dekapoden. Z. wiss. Zool. **121**: 1-63.
- Keim, W. 1915. Das Nervensystem von *Astacus fluviatilis* (*Potamobius astacus* L.). Ein Beitrag zur Morphologie der Dekapoden. *Ibid.* **113**: 485-545.
- Maynard, D.M. 1960. "The Physiology of Crustacea" (edited by T.H. Waterman). Academic Press, New York, I: 182-190.
- Miller, F.R. 1910. On the rhythmical contractility of the anal musculature of the crayfish and lobster. J. Physiol. **40**: 431-444.
- Muramoto, A. and K. Murayama 1965. The structure and the reflex mechanism of the PD organ of the crayfish, *Procambarus clarkii* Girard. Zool. Magazine **74**: 216-225.
- Orlev, J. 1926. Die Innervation des Darmes des Flusskrebsees. Z. mikr. anat. Forsch. **4**: 101-145.
- Otsuka, N. 1962. Histologisch Entwicklungsgeschichtliche Untersuchungen an Mauthnerzellen von Fischen. Z. Zellf. mikr. Anat. **58**: 33-50.
- Pringle, J.W.S. 1956. Proprioception in *Limulus*. J. Exp. Biol. **33**: 658-667.
- Prosser, C.L., R.A. Nystrom and T. Nagai 1965. Electrical and mechanical activity in intestinal muscles of several invertebrate animals. Comp Biochem. Physiol. **14**: 53-70.
- van Harreveld, A. 1936. A physiological solution for freshwater crustaceans. Proc. Soc. Exp. Biol., New York **34**: 428-432.
- Whitear, M. 1960. Chordotonal organs in Crustacea. Nature, Lond., **187**: 522-523.
- Wiersma, C.A.G. 1959. Movement receptors in decapod Crustacea. J. mar. biol. Ass. U.K. **38**: 143-152.
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